REMARKS

Claims 1-17, 19-53, 61, and 63 are pending. Claims 2, 7-13, 15, 25-31, 35, 36, 38, 40, 41, and 43 are withdrawn from consideration, leaving claims 1, 3-6, 14, 16, 17, 19-24, 32-34, 37, 39, 42, 44-53, 61, and 63 subject to examination. New claims 83-88 have been added. Claims 19 and 20 are rejected under 35 U.S.C. § 112, second paragraph for indefiniteness, and claims 1-17, 19-34, 37, 39, 42-53, 61, and 63 are rejected under 35 U.S.C. § 103(a) for obviousness. The rejections are addressed below, after a description of the amendments made herein.

The Amendments

As shown above, claim 1 has been amended to read as follows:

> 1. An osteoconductive and osteoinductive multilayer composite material comprising:
> I) an inner matrix being a composite material and comprising:
> (i) hyaluronic acid and hyaluronic acid derivatives,
> (ii) demineralised bone and/or biocompatible and biodegradable ceramics and/or bone of autologous or allogenic or animal origin, said matrix being sandwiched between
> II) at least two layers comprising a hyaluronic acid derivative superimposed on said inner matrix, said hyaluronic acid derivative being in a form selected from the group consisting of non-woven material, woven material, compact membrane or film, and perforated membrane or film.

Therefore, the expression "a matrix of" has been deleted from subpart (ii), in order to remove any possible misinterpretation, since the term "matrix" is also used in the second line of claim 1.

Similarly, the designations "A)" and "B)" have been substituted by the terms "I)" and "II)," respectively, in order to remove any possible misinterpretation, since "A)" and "B)" are also used in claims 3 and 21 with reference to the hyaluronic acid derivatives.

The term "external" has also been deleted, in order to overcome the rejection under 35

U.S.C. § 112, second paragraph (see below). Since the matrix (I) is specified as being "inner," with the at least two layers (II) superimposed on the latter, a multilayered material is obtained having the matrix always between the layers (II). This arrangement is made further clear by specification of the inner matrix as being "sandwiched between at least two layers" (II).

Furthermore, the hyaluronic acid (HA) derivative of the at least two layers (II) is now specified as being in a form selected from the group consisting of non-woven material, woven material, compact membrane and film, and perforated membrane and film. Support for this amendment can be found, for example, in paragraphs [0118-0120] of the published application (US 2007-0110819 A1), as well as in claim 32, now cancelled.

Certain dependent claims have been amended for consistency with currently amended claim 1.

Moreover, new claims 83-88 have been added, and support for these claims can be found at, for example, paragraph [0134] of the published application, as well as in Example 7.

No new matter has been added by the amendments.

Rejection under 35 U.S.C. § 112, second paragraph

Claims 19 and 20 were rejected under 35 U.S.C. § 112, second paragraph for indefiniteness, with the Examiner stating that it is unclear whether the layers in claims 19 and 20 are descriptive of either of the recited terms in claim 1, or if they refer to a third element of the composition. In response, Applicants submit that, as noted above, claims 19 and 20 each now designate the specified layers by reference to "II," clearly showing that the layers specified in claims 19 and 20 are the layers noted in section II of claim 1. Applicants further note that the Examiner expresses a concern that, because claim 1 includes open language as to the components

of the inner matrix and the layers, it is possible that the matrix and layers are of identical composition. In response, Applicants note that the specification of the composite material as being "multilayer" makes it clear that the inner matrix and layer components are distinct components of the claimed material. In view of the above, Applicants request reconsideration and withdrawal of this rejection.

Rejection under 35 U.S.C. § 103(a)

Claims 1-17, 19-34, 37, 39, 42-53, 61, and 63 are rejected under 35 U.S.C. § 103(a) for obviousness over Valentini et al., U.S. Patent No. 5,939,323.

This rejection has been met by the present amendment, in which the product of claim 1 is specified by the provision that the inner matrix (I) comprises hyaluronic acid **and** hyaluronic acid derivatives, with the matrix being **sandwiched between** at least two layers comprising a hyaluronic acid derivative in the form of non-woven material, woven material, compact membrane or film, or perforated membrane or film.

Applicants disagree with the Examiner's position in this rejection and, in support of the non-obviousness of the pending claims, hereby submit the following arguments, as well as a Declaration showing a comparison of a composition of the present claims with one made according to the methods of Valentini et al.

The Examiner states that in Valentini et al. the "scaffold-forming composition may be further modified by combining the HA derivative with a second phase, including hydroxyapatite or tricalcium phosphate (column 3, §3) and further including coating the composition with a second polymer (column 10, §(2)(f))."

Applicants traverse this reasoning, since the Examiner arbitrarily isolated sentences

from Valentini et al. in order to conveniently lead to the claimed invention. This is a clear case of **hindsight reconstruction of the claimed invention, which is not permissible.**

As a matter of fact, Valentini et al. in Example 1, column 10, §(2)(f) teach that: "In some experiments, HYAFF scaffold was also sprayed, dipped or coated with a second polymer including HYAFF, PLLA, PGA, etc., that contained a drug or peptide. This enabled release of the drug and tailoring of scaffold degradation rate."

Therefore, the scaffold of HA derivative obtained by Example 1 of Valentini can be sprayed, dipped, or coated with a second polymer. However, a skilled person undoubtedly would understand that the second polymer is intended to seep into the scaffold through the pores, irrespective of being sprayed, dipped, or coated. This is clear also when considering that the second polymer is designed to carry a drug or peptide. As a matter of fact, at column 6 of Valentini it is stated that a variety of materials can be used as coating. Particularly, at lines 35-38, it is reported that "the scaffold is coated with bone morphogenetic proteins (BMPs) or growth and differentiation factors (GDFs) in order to induce the formation of differentiated bone cells from bone precursor cells" (emphasis added).

Since the teaching of Valentini et al. is that **it is essential to have interconnected pores in the scaffold** (claim 1) **in order to allow the growth of cells into the scaffold,** "preferably penetrating the scaffold with cells" (column 4, lines 32-34), **the skilled person**, at the time of the invention, **was unavoidably led to assume that,** in order to induce the formation of differentiated bone cells from bone precursor cells within the interconnected pores, **the second polymer must necessarily reach the pores as much as possible.** Therefore, the coating, as well as the dipping and the spraying, has to be considered in this regard.

The current invention, particularly as specified in amended claim 1, refers to an inner

matrix on which at least two layers (II) comprising a HA derivative are superimposed, the HA derivative being in the form of non-woven material, woven material, compact membrane or film, or perforated membrane or film. This unequivocally means that the at least two layers are placed or laid on the surface of said inner matrix, so as to form the claimed multilayer material. The latter could have never be obtained by following the teaching of Valentini et al., because in the current invention **the at least two layers are clearly technically incompatible with a seeping step into the matrix**.

Furthermore, it should be noted that **the possibility of spraying, dipping, or coating the scaffold according to Valentini et al. is expressly taught as an alternative of "two-phase scaffold"** at column 6, lines 48-52. In fact, it is stated "Instead of coating the scaffold with the foregoing polymer materials a two-phase scaffold may be prepared, in which the scaffold **pores may be filled** with the foregoing materials or a hydrogel or ceramic. The two-phase scaffold may be prepared as described below in Example 5" (emphasis added). In particular, at the subsequent paragraph of the same column, lines 55-62, it is taught that:

> As mentioned above, the materials may be non-covalently coated on the scaffolds or covalently attached to the scaffolds. If covalently attached to the scaffolds, such covalent attachment may be carried out prior to the formation of the scaffold or may be carried out after formation of the scaffold. Drugs may be incorporated in a gel which solidifies within the scaffold (e.g. collagen type I).

This further confirms that **the coating has to be intended as filling of the interconnected pores**, such as by dip-coating (column 10, item a), line 10).

As far as the alternative of a **two-phase scaffold** is concerned, the only embodiment disclosed can be found at Example 5, as above cited.

In that Example, agarose microspheres were added to an HA derivative dissolved in

DMSO. The resulting sponge of HA derivative had the microspheres filling part of the interconnected pores of the obtained scaffold. At lines 30-34 it is stated that "the resulting scaffold contained entrapped microspheres which constituted 40-90% of the scaffold volume. 70-90% of the void volume contained microspheres with the remainder being empty due to microsphere loss."

Thus, the skilled person, at the time the invention was made, definitely and unambiguously knew from Valentini et al. that:

1) the scaffolds must be made of an HA derivative having "interconnected pores which permits cell ingrowth and, eventually, tissue replacement of the scaffold" (column 2, lines 54-56), "preferably penetrating the scaffold with cells" (column 4, lines 32-34);

2) the scaffold of HA derivative can be sprayed, dipped, or coated with a second polymer including HYAFF, PLLA, PGA, etc., that contained a drug or peptide, so that **said second polymer can reach said pores as much as possible**;

3) the possibility of **spraying, dipping, or coating the scaffold is expressly taught as an alternative to a "two-phase scaffold,"** wherein the scaffold pores may be filled with the foregoing materials or a hydrogel or ceramic, similarly carrying drug or peptide;

4) **the resulting scaffold**, either when sprayed, dipped, or coated, or when filled with microspheres, **is a single layer matrix**;

5) the only embodiments tested for cell growth within the designed porosities are scaffolds made of the sole HA derivative, i.e., **one-phase scaffold**.

From the above-described essential features for Valentini et al., it is evident that the skilled person is taught **to always configure a scaffold made of a single HA derivative having interconnected pores** where the ingrowth of cells takes place in order to succeed in repair

damage also to visceral organs, **wherein drug or peptide can be added by means of a second polymer carrying the same inside the pores.**

Furthermore, the fact that Valentini et al. refer to the possibility of "any shape and size" cannot in any way affect the provision of being a **one single layer scaffold, either having only one phase or two phases.**

Conversely, Applicants have surprisingly found that a multilayered composite material comprising an inner matrix (I) comprising:

(i) hyaluronic acid **and** hyaluronic acid derivatives, **and**

(ii) demineralised bone and/or biocompatible and biodegradable ceramics and/or bone of autologous or allogenic or animal origin, **said matrix being sandwiched between** at least two layers (II) comprising a hyaluronic acid derivative superimposed on said inner matrix, said HA derivative being in the form of non-woven material, woven material, compact membrane or film, or perforated membrane or film, allows obtaining **substitutes/grafts of bone tissue that are both osteoconductive and osteoinductive**, therefore able to induce the process of osteogenesis (see paragraph [0074] of the published application).

The current invention thus clearly differs from what is disclosed by Valentini et al., mainly in view of the following aspects:

- the matrix (I) is not required to have interconnected pores;
- the matrix (I) comprises (i) hyaluronic acid **and** hyaluronic acid derivatives **and** (ii) demineralised bone and/or biocompatible and biodegradable ceramics and/or bone of autologous or allogenic or animal origin, and therefore is not only a sole hyaluronic acid derivative;
- the final product is a multilayer composite material wherein the matrix (I) is

sandwiched between at least two layers (II) comprising a hyaluronic acid derivative; as clearly explained in the description, **each of these at least two layers (II) is a solid and discrete layer**, wherein the HA derivative concerned is in the form of non-woven material, woven material, compact membrane or film, or perforated membrane or film.

Thus, **what a skilled person was actually aware of is most certainly different from what was arbitrarily reconstructed by the Examiner having knowledge of the claimed invention**.

Further, Applicants submit that all of these structural differences, ascribable to the selected combination of features of the claimed multilayer material, result in a number of unexpected and advantageous technical effects.

In order to make the latter evident, Applicants enclose herewith a **Declaration of Ms. Anna Zanellato**, which includes a comparison of technical results obtained by carrying out the teachings of Valentini and the current invention.

Particularly, it has been the object of the experimentation described in the Declaration to determine the osteoinductivity and osteoconductivity of the claimed multilayer composite material. Particularly, a **matrix comprising HA, an HA derivative and DM** (demineralised bone matrix) is **sandwiched between two HA derivative layers**.

Experimental samples were prepared in order to assess osteoinductivity and osteoconductivity in vivo. When implanted into normal animals, human DM is xenogeneic, and is expected to provoke an immune response that may compromise the analysis of osteoinduction. To avoid this, the athymic mouse model was used. The athymic mouse lacks a thymus gland and, therefore, cannot mount a humoral immune response to the human DM implants.

A total of 18 samples were implanted bilaterally into mouse hamstring muscle (see Table below). The hamstring muscle group (biceps femoris muscle) is a large, easily accessible muscle, which is commonly used as an implant site to evaluate heterotopic bone formation. Histological evaluation of the test articles was conducted at 28 days after implantation.

Specifically, the claimed multilayer composite material was made according to **Example 7 of the invention**, therefore, wherein:

- the inner matrix (I) was a paste obtained with **Hyaff11p75 fibers** (benzyl ester of HA having a percentage of esterification of 75%, see paragraph [0134]), **hyaluronic acid** and **DM**;
- the matrix (I) was sandwiched between two layers (II) of **Hyaff11** (benzyl ester of HA, see paragraph [0134]), both in the form of a **woven fabric**;
- the so obtained multilayer material was calendered, freeze-dried, and then cut to size;
- the edges of the freeze-dried pieces were wetted with a solution of Hyaff11 in DMSO, and the process was continued to obtain the final dried multilayer composite material according to current claim 1.

Separately, a sponge of Hyaff11p75 was prepared according to **Example 1 of Valentini et al.**, the sponge further entrapping DM within its pores, in the same amount and from the same lot as of the DM used for producing the claimed product as described above.

Additionally, heat inactivated DM putty was provided as a **negative control**, the DM being of the same amount and from the same lot as the DM used for producing the claimed product and the product made according to Valentini et al., as described above.

A total of 18 samples were implanted and analyzed, wherein:

- Group **F1** included 8 samples of multilayer composite material according to claim 1;
- Group **F2** included 5 samples of the sponge according to Valentini et al.; and

• Group **F3** included 5 samples of negative control material.

Osteoinductivity, i.e., the capability to produce heterotopic bone de novo, and **osteoconductivity**, i.e., the capability to favor cell migration within the scaffold, were assessed histologically, by following intramuscular implantation of the samples in an athymic mouse model.

Each sample weighed approximately 20 mg. The samples were randomized and implanted bilaterally in the hamstring muscles of athymic mice. Intramuscular implantation of multilayer composite material of the invention is expected to induce cartilage and then bone formation within the implants. Animals were sacrificed at 28 days post-implantation.

Decalcified histology was then performed on the explanted samples; 5 histological slides with at least 2 sections per slide were prepared for each sample (at least 10 sections total per sample). Slides were stained with hematoxylin and eosin and a semi-quantitative scoring system was utilized to assess osteoinduction and osteoconduction using the scoring system described below.

The observer was blinded to the identification of the implant.

Osteoinductive and osteoconduction scores were based on the degree to which new bone, bone cells, osteoid, calcified cartilage remnants, and marrow elements are present.

The following scoring system was utilized:

0	No evidence of new bone formation
1	1-25% of the section is covered by new bone
2	26-50% of the section is covered by new bone
3	51-75% of the section is covered by new bone
4	>75% of the section is covered by new bone

The overall score for each implant was obtained by averaging the highest 5 scores from the histological slides; scores for each experimental group were determined by pooling the overall scores of the individual implants.

The results of semi-quantitative scoring are presented in Table at page 5 of the Declaration, from which it can be seen that the samples of multilayer composite material according to the invention (**F1**) were osteoinductive and osteoconductive, with an average bone score of **2.0**.

Conversely, the samples of sponge according to Valentini et al. (F2) had a definitely unsatisfactory average bone score of 0.24, even considering that the negative control DM (F3) produced no bone, with bone scores of '0'.

Particularly, it should be noted that, for the sponges of Valentini et al., the HYAFF polymer was visible in the histology slides, indicating that the polymer had not completely resorbed by 28 days post-implantation in the muscle pouch. Indeed, inflammation and no bone formation in the HYAFF polymer-rich regions of the sponges has been observed.

As far as the multilayer composite material according to the invention (**F1**) is concerned, Figure 1 and Figure 2 (pages 7 and 8 of the Declaration) have been enclosed in order to demonstrate the **surprising and advantageous osteoinductivity and osteoconductivity so achieved**.

Particularly, Figure 1 shows a DM-rich region in the sample with the highest bone score. It should be noted that **the formation of new bone ossicle with marrow** (arrow) **associated with residual DM**.

This confirms with no doubt that, if the skilled person had followed what is disclosed in Valentini et al., **even substituting the microspheres of agarose with DM**, they would have

never achieved the surprising results obtained with the multilayer composite material of the invention.

As a matter of fact, not only was **there no motivation or suggestion to search in the direction of the current invention** starting from Valentini et al., but also, **even if they considered to modify the teaching of Valentini et al., the skilled person would have at most considered to try a "two-phase" scaffold in which the agarose microspheres are substituted with DM as performed in the annexed Declaration. However, any expectation of success fell flat due to the definitely unsatisfactory results achieved and observed in that case.**

In fact, as above-mentioned, **the structural differences** of the multilayer composite material according to the invention, **in combination with the claimed selection of components,** allow obtaining substitutes/grafts of bone tissue that are **both significantly osteoconductive and osteoinductive**.

It should be pointed out that **Valentini et al. only observed osteoblast ingrowth within the porous scaffold when the latter was in the form of sponge of a single phase** (Examples 2 and 3). The annexed Declaration shows that, by following the teaching of a "two-phase" scaffold, the resulting matrix does **not** shows acceptable osteoinductivity and osteoconductivity.

Therefore, **a skilled person would have been even led to believe that a single-phase scaffold can perform better than a two-phase scaffold**, when the technical effect of significantly enhanced osteoinductivity and osteoconductivity is desired. As a consequence, **how could the skilled person have considered, not only to design an inner matrix such as the claimed one, but also to sandwich such a matrix between at least two additional layers having the claimed features, for significantly increasing osteoinductivity and**

osteoconductivity, when Valentini et al. lead to the contrary?

Besides this clear **teaching away**, Applicants have found that a multilayer composite material such as that of the invention, having the following structural particulars:

- the matrix (I) is not required to have interconnected pores;
- the matrix (I) comprises (i) hyaluronic acid **and** hyaluronic acid derivatives **and** (ii) demineralised bone and/or biocompatible and biodegradable ceramics and/or bone of autologous or allogenic or animal origin;
- the final product is a **multilayer composite material wherein the matrix (I) is sandwiched between at least two layers (II)** comprising a hyaluronic acid derivative; the at least two layers (II) being a solid and discrete layer, wherein the HA derivative concerned is in the form of non-woven material, woven material, compact membrane or film, or perforated membrane or film,

allows **very appreciably** obtaining a product being **both significantly osteoconductive and osteoinductive**.

For all of the reasons described above, Applicants submit that the claimed invention is new and **non-obvious** over Valentini et al., by having proved the **objective criticality of the claimed multilayer structure together with the selected combination of components**. The demonstration has been carried out by comparing the prior art product in the embodiment, among the embodiments of Valentini et al., repeated in an embodiment **not explicitly disclosed but closer** to the product of the current invention, in order to **undoubtedly and unequivocally highlight the absolutely different technical effects** respectively achieved, as also suggested by the Examiner at the end of page 7 of the outstanding Office Action.

CONCLUSION

Applicants submit that the claims are in condition for allowance, and such action is respectfully requested. If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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